# Relationship between tissue carotenoid concentrations in the Pacific mole crab, *Emerita analoga*, and parasitism by the acanthocephalan, *Profilicollis altmani*

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# Approval Page

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**ABSRACT**

Acanthocephalan worms infect two hosts to reach full development. Some species have been demonstrated to change their intermediate host's behavior or coloration to increase the likelihood they will reach the final host. The pacific mole crab, *Emerita analoga,* is commonly parasitized by *Profilicollis altmani* along the Pacific coast of North and South America, but the impact of parasitism on the crab intermediate host is unclear. We investigated whether *P. altmani* alters the concentration of dietary carotenoids present in *E. analoga* tissues and if the parasite may also acquire pigments normally allocated to the host. Fifty eight gravid female crabs were collected and dissected during the summer of 2008 from Pismo Beach, California. Ovaries, eggs, esophagus, and carapace, as well as cystacanth stage acanthocephalans, were removed from each crab. The samples were weighed and extracted in HPLC grade acetone. Carotenoids extracted from each tissue type were analyzed by UV-vis spectrophotometry, with the maximum absorbance and maximum wavelength recorded for each sample. Results were compared to the wavelength and absorbencies of previously identified carotenoids in *E. analoga*. The carotenoid content of each tissue type with respect to parasite load group was compared. Host carotenoid profile patterns differed between tissues and some with respect to parasite loads. Carapace and ovary tissue had differences between some absorbance groups, but as the data were not normally spread due to uneven sample sizes, the results cannot be confirmed. Cystacanths found in infected crabs contained carotenoids, but their UV-vis profile appears to differ from that of host tissues. This suggests that the parasites may modify host-acquired carotenoids.

## INTRODUCTION

The sand crab or Pacific mole crab, *Emerita analoga*, is found on coastlines ranging from Oregon to Panama and is very well adapted to living on open beaches. They tend to move up and down the beach with the tide for ease of feeding. They are filter feeders that obtain carotenoids from their plankton diet. Sand crabs are also known for burying themselves deep in the sand primarily to evade predators such as sandpipers, sanderlings, godwits, black-bellied plovers, Willits, curlews, scoters, and other crabs. The mating season is between May and July during which male crabs are more populous than female crabs. Female crabs are noticeably larger than male crabs and are generally more populous than male crabs with the exception of mating season (MacGiniti, 1938). They play an important part in balancing the California coastline's ecosystem by ingesting plankton, supporting acanthocephalan parasites, and acting as prey to many shore birds.

The Acanthacephala are a phylum of parasitic worms characterized by their proboscis that is used to penetrate the host digestive tract wall. After fertilization of the zygote the acanthor, the stage that is infective to the intermediate stage, is formed. The acanthor then exits the mother's genital pore to be released in the environment. The acanthor undergoes development of the proboscis outside the mother and matures into a cystacanth, or juvenile. The cystacanth is capable of infecting the definitive host and completes development in the intermediate host. Once development is complete the cystacanth must be ingested and mature inside the definitive host to complete development. Often the cystacanths end up in a non-definitive host in which they can not complete development into an adult; these accidental hosts are termed paratenic

hosts and the parasites often cause fatal damage to their paratenic host (Crompton and Nickol, 1985).

Along the Pacific Coast from Alaska to Baja California, *Emerita analoga* serves as the intermediate host for *Profilicollis* spp. acanthocephalans. At least one species of *Profilicollis* is known to infect *E. analoga*, *Polymorphus altmani* (thought to be synonymous with *P. kenti*), though more are yet to be identified (Crompton and Nickol, 1985). The final host for these parasites is often shore birds such as gulls and Surf Scoters (*Melanitta perspicillata).* The paratenic host for *Profilicollis* spp. is often sea otters, *Enhydra lutris* (Crompton and Nickol, 1985). When infected the sea otter's digestive tract is often damaged from the spiny proboscis and oftentimes the animal dies as a result.

Historically, *Polymorphus* and *Profilicollis* were thought to be two separate subgenera of Acanthocephala based on differences concerning the shape of the proboscis, length of the neck, and whether or not the fibrillar coat of the egg possessed polar swellings. Schmidt and Kuntz (1967) argued however that the long neck and spheroid proboscis characteristic of *Profilicollis* also occurred in some degree in some *Polymorphus* species. Furthermore, they concluded that polar swellings of the fertilization membrane are too generic of a trait to be a characteristic of one genus. Nickol and Crompton (1999) identified *Polymorphus* parasites from the crustacean order Decapoda and concluded them to be synonymous with the *Profilicollis* genus. Among these synonymous species includes *Polymorphus altmani* and *Polymorphus kenti*, both of which were found in *Emerita analoga*. From the parasites found and identified and due to the spherical-to-ovid proboscis, long neck, and concentric egg membranes it was concluded that all known

records of *Polymorphus* in decapods refer to species of *Profilicollis* (Crompton and Nickol, 1999).

As there is no known de novo carotenoid synthesis in any animal, parasites that contain carotenoids must have obtained the carotenoids from their hosts. Analysis of six species of adult acanthocephalans revealed that each species contained a single major carotenoid; *Macracanthorhynchus hirudinaceus* (from the small intestine of the pig, *Sus scrofa*), *Pseudoacanthocephalodies galaxis* (from the intestine of small galaxid fish from New Zealand) and *Neoechinorhynchus pseudemydis* (from the Map turtle, *Pseudemyscripta elegans*) all contain primarily lutein, both *Pomphorhynchus laevis* (from the intestine of chub, *Squalius cephalus*) and *Filicollis anatis* (from the common eider, *Somateria mollissima mollissimia*) contain primarily β-carotene, and *Nipporhynchus ornatus* (from the Skipjack tuna, *Katsuwonis pelamis*) contains primarily esterified astaxanthin (Barrett and Butterworth, 1973). The function of carotenoids in adult acanthocephalans is unknown, though carotenoids are thought to function as anti-oxidants and be involved in oxidative metabolism and reproduction. The authors also noticed that the carotenoids found in the adult acanthocephalans did not reflect the spectrum of carotenoids available to the parasite from the finals host's diet. This leads to the conclusion that the acanthocephalans selectively absorb carotenoids from the host, firstly because they contain primarily one carotenoid and secondly, the one carotenoid they contain is not necessarily highly concentrated in the host (Barrett and Butterworth, 1973). Thus the parasite likely selects for the one carotenoid they maximally absorb. This begs to question if parasites also absorb carotenoids from their intermediate host, not solely the final host.

Previous studies of *E. analoga* have concentrated on identifying all carotenoids present in the crab. Using a combination of methylation, absorption spectra, and thin layer chromatography, eight carotenoids were identified from *E. analoga* ovaries, eggs, carapace, and hemolymph; α-carotene, β-carotene, echinenone, canthazanthin, zeaxanthin, diatoxanthin, alloxanthin, and astaxanthin. The highest concentrations of carotenoids were found in august, and in general females had a higher concentration than males (Gilchrist and Lee, 1971). Curiously, no parasites were mentioned; either they were ignored or it was unclear to the experimenters what they were. To date there have been no studies on the possible uptake of *E. analoga* carotenoids by *Profilicollis* acanthocephalan worms. It is unknown whether or not the parasites selectively or passively absorb carotenoids from *E. analoga*, or if they absorb carotenoids at all. Furthermore no studies have been performed to see the results of an acanthocephalan infection upon the tissues of the crabs, specifically the esophagus, carapace, eggs, and ovaries. To date no concrete functions of carotenoids have been identified though it is possible they serve as anti-oxidants (Gilchrist and Lee, 1971); other experiments have suggested they participate in photochemical processes, electron transport, or enzymatic reactions (Cheesman et al., 1967).

Acanthocephalan cystacanths are white ovid shapes about 2mm in length and can usually be seen with the naked eye in the hemocoel of an infected crab, near the midgut. Acanthocephalans are reputed to change their intermediate's host behavior but it is unknown if they change that of *Emerita analoga*. Furthermore, if the parasite does indeed change a behavioral or physical aspect of any individual crab the end consequence was unknown. Upon preliminary dissections of infected crabs by one of the research members, Lauren Constancio, it was observed that the parasites often had an orange-pink hue and were sometimes found in the ovaries of the crab. This

was unexpected, as the eggs are known to be swallowed by the crab and assumed to complete their development into the cystacanth in the intestine of the crab (Crompton and Nickol, 1985). Also during the preliminary dissections the esophagus was also identified. It appears as a bright red dot of tissue.

Our hypothesis is that the developing parasites steal carotenoids from the intermediate host crab. This is hypothesized to serve one of two purposes: first, that the carotenoids aid the parasite in development, and second, that the decreased concentration of carotenoids is detrimental to the crab's fitness and may result in the increased predation of the crab by the parasite's definitive host. We will dissect collected crabs and extract the carotenoids present in each tissue. By doing UV-visible spectroscopy on each tissue we will look at the maximum absorbance and the wavelength at the maximum absorbance to determine which carotenoids are present in each tissue (all carotenoids have a characteristic wavelength they maximally absorb) and if a high parasite infection correlates with a lower absorbance of tissue samples.

## MATERIALS AND METHODS

### MATERIALS

Dissecting microscope, Petri dish, scissors, tweezers, scalpel, plastic Ziploc bags, 1.5 mL microcentrifuge tubes, latex gloves, de-ionized water, digital calipers, transfer pipettes, micropestles, mortar and pestle, HPLC grade acetone, metal spatula, sharpie, labels, scotch tape, paper, microcuvettes, cuvettes, UV-vis spectrophotometer, computer, printer, Microsoft excel, foil, liquid nitrogen, centrifuge, vortexer, microscale, fume hood (for the initial extraction

procedure the following was needed in addition to the materials above: 100% NaCl, 1M acetone, anhydrous ethyl ether, sodium sulfate drying column, separatory funnel, and a 15mL tube).

## METHODS

A total of 58 gravid female crabs were collected between the months of July and August of 2008. Each crab was dissected and the tissues partitioned out for the carotenoids to be extracted at a later date. Once the carotenoids were extracted UV-visible analysis was used on ovary, egg, esophagus, and carapace samples; unfortunately not all tissues were salvaged from each female so the samples sizes between tissues are not even.

**Collection and Dissection** *Emerita analoga* were collected from Pismo beach, Avila beach, and Montana de Oro, on the central coast of California. *E. analoga* were collected from the months of January through September of 2008. After collection, they were bagged, labeled (according to the beach they were found at and a number they were assigned), and stored in a -20°F freezer until they were dissected. Before dissection, the sex (females were identified by the presence of pleopods or eggs) and carapace length was recorded (mm) using digital calipers. *E. analoga* was placed in a Petri dish with a small amount of deionized water. The carapace was cut down the middle on the dorsal side of the crab and peeled away with tweezers. The gonads, digestive gland, hindgut, eggs (from gravid females) and esophagus were separated from the crabs using a dissecting microscope. Each tissue, including the carapace, was placed and labeled in a 1.5 mL microcentrifuge tube. All cystacanths (acanthocephalan juveniles) found in *E. analoga* were

collected in a 1.5 mL microcentrifuge tube and saved as well. The tissues and parasites were stored in a - 80°F freezer until extractions of the sample carotenoids were performed.

**Initial Extraction Procedure** The samples were homogenized with liquid nitrogen, vortexed with 1M acetone, and centrifuged. The supernatant was pulled off into a 15mL tube and the pellet was re-suspended in acetone until it appeared white. The sample was centrifuged again and both supernatants were pooled. 1 mL ethyl ether was added, the solution mixed, and 1 mL of 100% NaCl added. The phases were separated using a separatory funnel and the organic phase was dried on a sodium sulfate drying column. The extracted carotenoids were amassed in a 1.5 mL microcentrifuge tube and stored in the dark.

**Final Extraction Procedure** After preliminary results determined the initial procedure did not yield ideal results a new procedure was adapted. The samples extracted with the initial procedure were still used for results once the ethyl ether was evaporated off and the carotenoids re-suspended in HPLC grade acetone. Depending on which tissue sample was being extracted, the tissue was weighed out according to a set weight; egg samples were 0.05 grams, esophagus samples were 0.002 grams, ovary samples were 0.01 grams (the concentration was 0.0066g/1 mL acetone; some of the ovary samples were smaller than 0.01 grams so enough acetone was added to make a dilution of 0.066g/mL), and carapace samples were 0.05 grams. All samples but carapace samples were wet weights. The carapace samples were placed in mortars and enough liquid nitrogen was added to blanket the sample; the sample was ground into a dry powder with a pestle until the liquid nitrogen completely evaporated. Once the sample was a dry powder, the required weight of sample was added to a 1.5 mL microcentrifuge tube. For esophagus, egg, and

ovary samples, the sample was pulverized in its tube using a micropestle. Once the sample was uniform in consistency, the correct amount of tissue was weighed and placed in a new 1.5 mL microcentrifuge tube (all tissue not used due to the weight limit was stored back in the - 80°F freezer). Once the samples were correctly weighed and in a new 1.5 mL microcentrifuge tube 1 mL of acetone was added. The tube was shaken and vortexed briefly several times. The sample was centrifuged for 30 seconds on high. The supernatant was removed and collected into a new 1.5 mL microcentrifuge tube using a transfer pipette. Next, 0.5 mL of acetone was added to the pellet and the pellet was re-suspended using a metal spatula. The sample was vortexed and centrifuged again. The supernatant was removed again and added to the first supernatant. The pellet was discarded. The final product was a 1.5 mL sample of extracted carotenoids. The extracted samples were stored in the - 80°F freezer until UV-vis spectroscopy was performed. Until then, the samples were kept in the dark by covering the samples with foil.

**UV-vis spectroscopy** The tissue samples for each crab were examined using UV-visible spectroscopy. The room was kept as dark as possible and samples wrapped in foil until processing. HPLC grade acetone was used as a blank for the spectrophotometer and to rinse and clean out used microcuvettes. Each sample was pipetted using a clean transfer pipette into a microcuvette and the absorbance and wavelength was recorded. 1mL of each sample was pipetted into the cuvette. When this was not possible (again, in the case of some ovary samples the sample was smaller than 0.01 grams and the all the sample was measured out into the 1.5 mL tube. Enough acetone was added to make a 0.066 g/mL solution which in some cases yielded a smaller volume than 1 mL) a cube of paper 6mm in height was used underneath the microcuvette. The cube enabled the absorbance of the sample to be measured without hitting the

meniscus in the microcuvette and sensing erroneous values. The values were copied and pasted to a Microsoft excel worksheet. The samples were quickly pipetted back into the 1.5 mL microcentrifuge tube in order to decrease the amount of evaporation of sample. For all samples, the maximum absorbance and the wavelength (nm) at that absorbance was recorded using Microsoft excel.

## RESULTS

**Collection and Dissection** As it was nearing the end of the mating season when crabs were collected the females were more numerous and more of the female sex were collected. Upon preliminary dissection the cystacanths appeared as small white ovid shapes about 2-3mm in length. They had an orange-pink hue though it was unclear if they were actually colored or the tissue surrounding them was colored. Digestive tissue was brownish in color whereas ovarian tissue was more orange in color. The hindgut was a clear tube that was often hard to differentiate from the digestive gland. Due to difficulty in differentiating the hindgut and digestive gland those tissues were not used for spectroscopy. Furthermore, only the females collected from Pismo beach were used for UV-vis spectroscopy.

**Extraction** The first extraction procedure did not yield a high quantity or quality of carotenoids in solution so the second procedure was adapted. This gave a much higher yield of carotenoids for spectroscopy. The samples were also able to be used for HPLC measurements after the UV-vis results were finished.

**UV-vis Spectroscopy** There were no outstanding results that gave any specific conclusions from the graphs so bar graphs were constructed for each tissue to determine the difference in parasite load in groups of different absorbencies for the different tissues. These absorbencies were taken from visible breaks in the graphs for each tissue. In general however, there were some interesting trends present: wavelength at maximum absorption increases with absorption for carapace and esophageal tissue whereas it decreases for egg and ovarian tissue, wavelength at maximum absorption stays constant with carapace length for all tissues except esophageal tissue where it tends to decrease, wavelength at maximum absorption stays constant with parasite load for all tissues, maximum absorbance decreases with carapace length for all tissues but esophageal tissue where is increases, for all tissues parasite load increases with carapace length, and maximum absorbance decreases with parasite load for all tissues except esophageal tissue where it tends to increase. As indicated above esophageal tissue shows different trends than most of the other tissues.

**Carapace** The graphs alone do not offer much information, though some interesting trends are present; wavelength at maximum absorption stays relatively constant with parasite load (fig. 1.3 and 1.4) and carapace length (fig. 1.5 and 1.6), maximum absorption decreases with increasing carapace length (fig. 1.7), parasite load increases with carapace length (fig. 1.8), and maximum absorbance decreases with increasing parasite load (fig. 1.9). Interestingly, fig. 1.1 and fig. 1.2 show contrasting results; each display the relationship between wavelength at maximum absorption and maximum absorption but the trends are contradicting. The graph that includes the outliers (fig. 1.2) shows a negative correlation while the graph excluding the outliers (fig. 1.1) shows a positive correlation. The graphs do show four distinct groups in wavelength absorption

(fig.1.3): a) 45-476 nm, b) 466 nm, c) 480-485 nm, and d) 478-479.9 nm (fig. 1.10). Each group corresponds to one or more carotenoids (see discussion). The residual diagnostics test (summarized in table 6) that examined the relationship between wavelength and parasite load showed that the data followed a normal trend but that the data are not evenly spread (as is the case for all tissues excluding esophagus tissue). A Tukey analysis showed that groups B and C, and C and D have significant differences between them. Group B contains 10 samples, group C contains 24, and group D contains 16. The power analysis gave a maximum difference of 0.83; in other words, there is an 80% chance of seeing a difference if the difference is 6.7 fold. The null will be rejected in that there is a significant difference between groups B and C, and C and D.

**Ovary** The following trends are present: parasite load increased with carapace length (fig. 2.1), maximum absorbance decreased with increasing carapace length (fig. 2.2), wavelength at maximum absorbance remains constant with carapace length and parasite load (fig. 2.3 and 2.6), wavelength at maximum absorbance increases with decreasing absorption (fig. 2.4), and maximum absorption decreases with parasite load (fig. 2.5). Table 7 examines the relationship between the four groups of wavelength absorption identified in fig. 2.7 and their corresponding average parasite load: 454-454 nm (A), 455-457 nm (B), 470 nm (C), and 480-483 nm (D). A residual diagnostics test showed that the data follow a normal trend but the distribution between the four groups is not evenly spread: group C only has one sample, group B has 15, and group A has 21 samples. A Tukey analysis revealed that there are significant differences between groups A and B, and B and C. The power analysis showed a maximum difference of 0.30; hence there is an 80% chance of detecting a difference if the difference is 2 fold. Since the data have an uneven

spread, particularly group C, the significant difference between groups B and C may not be significant at all. However there may be a significant difference between groups B and A; the null will be rejected.

**Egg** Egg samples show the following trends: carapace length increases with parasite load (fig. 3.1), maximum absorbance remains constant with carapace length (fig. 3.2), wavelength at maximum absorbance remains constant with carapace length and parasite load (fig. 3.3 and 3.5), maximum absorbance decreases with parasite load (fig. 3.4), and maximum absorbance decreases with wavelength at maximum absorbance (fig. 3.6). Table 5 examines the relationship between the two groups of wavelength absorption identified in figure 3.7 and their corresponding average parasite load: A) 454-458 nm and B) 483 nm. A residual diagnostics test revealed that the data are normal but there is an uneven spread between the two groups; group A is much larger in number than group B. A Tukey analysis revealed that there is no significant difference between the two groups, and also that the P value is 0.80. The power analysis showed that at 0.8 power there is a maximum difference of .23; in other words (if the out put in un-logged) there is an 80% chance of detecting a difference if the difference was 1.7 fold. The null will not be rejected; there is no significant difference between the groups.

**Esophagus** The following trends are present: carapace length increases with parasite load (fig. 4.1), maximum absorbance increases with carapace length (fig. 4.2), wavelength at maximum absorbance decreases with carapace length (fig. 4.3), wavelength at maximum absorbance increases with maximum absorbance (fig. 4.4), wavelength remains constant with parasite load (fig. 4.5), and maximum absorbance increases with parasite load (fig. 4.6). Figure 4.7 shows that

like the other tissues there so some divisions in the wavelength at maximum absorption spectra. These divisions are shown and compared to each other with their average respective parasite load in table 8; A) 460 nm, B) 462 nm, C) 466 nm, and D) 470-480 nm. A residual diagnostics test was run, revealing that the data are normal and there is a normal spread between the four groups. A Tukey comparison revealed that none of the groups have a significant difference in parasite load, supported by the fact that the P value is 0.79. This indicates the null will fail to be rejected. A power analysis revealed the maximum difference at 0.80 to be 0.42; un-logging this number indicates that in order to detect any difference between the four groups the difference would have to be 2.6 fold.

## DISCUSSION

The range of absorbencies for each tissue is consistent with the absorbance range of the major carotenoids in *E. analoga*. The specific absorbance of each carotenoid varies depending on what it is extracted or suspended on. In general however, β-carotene absorbs around 450nm and occasionally 478nm (Shafaa et al., 2007), astaxanthin around 470-480nm (Britton, 1995 and Shafaa et al., 2007), canthaxanthin around 466-480nm (Britton, 1995), echinenone around 455-472nm (Britton, 1995), and zeaxanthin around 449-452 (Britton, 1995). The other carotenoids in *E. analoga* are minor carotenoids of the total 600 that exist in nature (Shafaa et al., 2007) and are difficult to obtain absorption spectra for. Furthermore, they are the less prominent carotenoids in *E. analoga* as far as total concentration goes (Gilchrist and Lee, 1971).

In crustaceans, carotenoids can exist in three forms: free pigments, such as carotenes, esterified xanthophylls, and xanthophylls attached to proteins, or carotenoproteins. Carotenoproteins are

frequently seen in the eggs, ovaries, hypodermis, carapace, retina, gut, and hepatopancreas. Esterified xanthophylls and carotenes are usually seen as storage forms. As seen in other experiments, astaxanthin makes up the bulk of the carapace, with some traces of canthaxanthin. The digestive tract is sometimes seen as a storage site for carotenoids, usually for both β-carotene and astaxanthin (Goodwin, 1980). The gonads also serve as a storage site for carotenoids, particularly during the reproductive season.

**General Trends based on UV-vis spectroscopy** The relative abundance of carotenoids in tissues of parasitized crabs was examined using UV-vis spectroscopy to determine whether or not highly parasitized crabs contain a lower concentration of carotenoids than lesser parasitized crabs; it was hypothesized that either the parasites selectively take the host's carotenoids for developmental advantages or for detrimental reasons to the host to increase risk of predation. In general, as carapace length increased, so did parasite load. Logically it makes sense that the larger crabs have a higher level of parasitism; they have lived longer and eaten more than small crabs. For carapace, ovary, and egg tissue as maximum absorbance decreased, carapace length and parasite load increased. This could indicate that acanthocephalans do absorb carotenoids from their intermediate host because a lower absorbance indicates a lower concentration of carotenoids for those crabs that are highly parasitized. Wavelength at maximum absorbance however stays constant with parasite load, giving no indication which carotenoids are absorbed from the crab host. For both carapace and esophagus, as wavelength at maximum absorbance increased, maximum absorbance increased; the opposite was true for ovary and egg tissue. This makes sense for carapace tissue because astaxanthin is the main carotenoid present in that tissue. For eggs and ovarian tissue it indicates that at higher concentration there is a lower absorbance,

indicating that β-carotene is present in high concentration in reproductive tissues. In addition, figure 2.7 shows that ovarian tissue has higher parasite loads at wavelength absorption between 455-457 nm. The fact that as maximum absorbance decreased as parasite load increased for carapace, ovary, and egg tissue would support the hypothesis that the parasites steal carotenoids for their host but it cannot be said with certainty that this is true.

**Carapace Tissue** As stated before, the major carotenoid present in the carapace is astaxanthin, with a notable increase in concentration as the reproductive period continues. It is, in general, the most abundant carotenoid present in *E. analoga* (Gichrist and Lee, 1971). There has been evidence this is because a majority of the carotenoids in *E. analoga's* diet can at some time be converted to astaxanthin. Through a series of oxidation reactions β-carotene can be converted into either echinenone or zeaxanthin; echinenone can further be converted to canthaxanthin and then astaxanthin while zeaxanthin can be converted right to astaxanthin (Goodwin, 1980). If this is happening in *E. analoga* it would be expected that many of the tissues (other than the eggs and ovaries) would have had a high concentration of astaxanthin and thus absorbed in the 470-480nm range. This could be an explanation for the trend in figure 1.1; it appears that as wavelength at maximum absorbance increases in carapace tissue maximum absorbance increases. This could be because as more pigment is absorbed more is oxidized to astaxanthin, which is why there is high absorbance around 480 nm. For carapace tissue there were significant differences in parasite load between groups B and C, and C and D (see figure 1.10). Group B is that which maximally absorbed at 466 nm, corresponding to canthaxanthin, group C absorbed around 480-485 nm, corresponding to astaxanthin and canthaxanthin, and group D absorbed around 478-479.9 nm, corresponding to β-carotene. Group D had the highest mean parasite load, group C followed, and

group B had the lowest mean parasite load. In other words, crabs containing high amounts of β-carotene also have the highest mean parasite load. This is significant because the group corresponding to β-carotene in carapace tissue has a higher mean parasite load than the other groups corresponding to astaxanthin and it would be expected that the carapace has more astaxanthin than β-carotene. This could mean the parasites either steal astaxanthin or they prevent the crabs from oxidizing the β-carotene they ingest.

Astaxanthin is the primary carotenoid in the carapace and is believe to have antioxidant properties that play a key role in protection against UV light potooxidation, inflammation, cancer, aging, enhancement of immune system, liver function, and heart, eye, joint, and prostate health (Ravishankar et. al, 2006). It is possible that astaxanthin is responsible for the carapace color due to the fact it plays a role in protection against UV light potooxidation. Thus, if the parasites are present in crabs with a lot of β-carotene it could be that those crabs have a different color than others. The parasites could take β-carotene, preventing the crab from converting it to astaxanthin, or the parasites could be attracted to those crabs who do not convert their β-carotene to astaxanthin as quickly as others. Either one of these options could, if indeed astaxanthin is responsible for carapace color, discolor the carapace of a crab, thus making it more susceptible to predation.

**Ovary** For ovary tissue there were significant differences between groups A and B (see figure 29). Group A was comprised of the absorbencies of 452-454 nm, corresponding to β-carotene and zeaxanthin; group B was comprised of the absorbencies 455-457 nm, corresponding to echinenone. Group A had a lower mean parasite load than group B, indicating that parasites may

take β-carotene or zeaxanthin (or another lower absorbing carotenoid) from crabs or that crabs with high levels of echinenone are preferentially infected by acanthocephalans. Again, zeaxanthin, echinenone, and β-carotene have all been known to be converted to astaxanthin (Goodwin, 1980). β-carotene is not known to increase or decrease egg development (Gilchrist and Lee, 1971) so there should not be any reason that decreased β-carotene is detrimental to ovarian tissue. Thus the difference between these groups could be coincidental or refer to something else. It is also possible that the ovary utilizes a specific carotenoid such as echinenone for development or benefit to the host but to date no research has supported this. Thus, there is no conclusive reason that ovarian tissue from highly parasitized crabs should be high in echinenone compared to crabs infected with low levels of parasites.

**Eggs** In the total amount of eggs collected there were both brown (late stage) and orange (early stage) eggs. Previous studies have shown a similar abundance and total concentration of carotenoids in both types of eggs. This suggests that the carotenoids present in the eggs are not utilized for egg development (Gilchrist and Lee, 1971). Indeed, it was found that there is no marked increase of carotenoid content in the eggs of *E. analoga* during embryogenesis; however there was a marked change right before the eggs hatched (Goodwin, 1980). In the egg tissue studied there were no significant differences found (see figure 30). However, there were high amounts of samples that absorbed around 454-458 nm, corresponding to β-carotene. There was a low sample size that absorbed at 483 nm, corresponding to astaxanthin and canthaxanthin. The highest proportion of β-carotene occurs in the ovaries and eggs in September; in addition, as the peak reproductive period is reached and passed there is an increase in astaxanthin in the eggs. There seems to be a parallel between β-carotene and astaxanthin production, indicating

astaxanthin production relies on a steady supply of β-carotene. Towards the end of the reproductive season there is a notable excess of astaxanthin in both the eggs and carapace (Gilchrist and Lee, 1971). In the eggs studied there was a low sample size that absorbed around the wavelength of astaxanthin. This could indicate that in the hatching process β-carotene is more important than astaxanthin and canthaxanthin. It is also possible the acanthocephalans steal astaxanthin from the eggs to damage host development. Again, there was no significant difference between the two groups so no conclusive evidence is supported.

**Esophageal Tissue** The trends for esophagus are interesting but as this tissue has not been studied in the past it is difficult to know what the trends mean. Esophagus tissue had two unique trends; as maximum absorbance increased, so did carapace length and parasite load. This could be because the esophagus gets the first carotenoids ingested in feeding; most likely an array of different carotenoids. Larger crabs eat more and attract more parasites; the more crustaceans eat the higher the concentration of carotenoids in their diet. Again, the esophagus is a bright red tissue and it could be that it absorbs any and all carotenoids that go through it. It could also be that that organ does not need particular carotenoids; thus it could absorb them and transfer them to the organs in need of carotenoids. Unfortunately no conclusive evidence was found that esophageal tissue was related to parasitism though it does show some interesting trends opposite of that of the other tissues.

Previous experiments have utilized UV-vis absorption spectra for characterization of carotenoids, not necessarily in crab tissue, but to identify the different carotenoids present in liposomes, liver microsomes, and retinal epithelial cells (Shafaa et al., 2007). According to the

Lambert-Beer law biomolecules absorb light at a characteristic wavelength. This is summarized in their equation:

$$Abs = \varepsilon lc$$

(where *Abs* is absorption, $\varepsilon$ is the extinction coefficient which is specific to each compound, and $l$ is the length that the light travels, usually the length of the cuvette) and provides evidence to the concept that absorbance is proportional to the concentration of the absorbing solution (Lodish et al., 2008). This means that the absorbance of each tissue for each crab is a measure of how concentrated the carotenoid is in solution. Performing UV-vis on solutions of carotenoids is complicated because the absorbance for a solution of carotenoids will be at an intermediate wavelength to the individual carotenoids. UV-vis merely measures chromophores of the carotenoid and conjugated carbonyl groups. Carotenoids in solution do follow the Lambert-Beer law but because there is a mixture of carotenoids in *E. analoga* tissues the exact carotenoid present in that tissue can not be definitively determined (although extinction coefficients are available for most carotenoids) (Britton, 1995).

Studies of the relationship between a host fish, *Tinca tinca*, and a parasite of the fish, *Ergasilus sieboldi*, have indicated the average mass carotenoids in parasitized fish are lower than in healthy unparasitized fish. Using thin layer chromatography the carotenoids in an array of tissues were evaluated in mass against tissues of unparasitized fish. The results showed that unparasitized fish have an average of 2.323µg of carotenoids per gram of live mass whereas infected fish have an average of 0.141µg/gram. Furthermore, the specific carotenoids present in tissues of infected fish

indicated that the parasite inhibits conversion of β-carotene into its carotenoid derivatives such as zeaxanthin and canthaxanthin (Czeczuga, 1980). Although *Ergasilus sieboldi* is not an acanthocephalan it does provide evidence that parasites do absorb carotenoids from their hosts.

In a repeat experiment it would be helpful to determine the average mass of carotenoids in the whole tissue of the crab. This would ideally help normalize the trend in sample size and allow more conclusions to be drawn based on carotenoid content of infected crabs in general. It would have also been useful had the parasites themselves had been extracted for carotenoids and examined with UV-vis spectroscopy. This would allow us to see if they contain the same carotenoids as the tissues they were found in and the crab in general.

Future studies could also concentrate on the importance of astaxanthin in the carapace and the esophagus in regards to carotenoid concentration. Identifying the carotenoids in the esophagus could provide insight into the diet and ingestion of particular carotenoids. In addition it would be interesting to determine if astaxanthin is indeed responsible for the color of the carapace. Performing UV-vis spectroscopy on carapace samples while comparing the shade and color of each one with the maximum absorption would provide information on the color of the carapace and how it relates to carotenoid content and also level of parasitism. It would be interesting to see the chromatic difference in those carapaces with higher β-carotene or astaxanthin concentrations. Also in future studies it would be easier to draw conclusions if the samples sizes were more uniform; in this study the only tissue in which the group size was uniform was the esophagus. There were also some extreme outliers (especially with the carapace tissue) which skewed the results.

## CONCLUSION

For carapace, ovary, and egg tissue, that maximum absorbance showed a decrease when parasite load increased. This could indicate that acanthocephalans do absorb carotenoids from their intermediate host. As absorbance is proportional to concentration, a lower absorbance indicates a lower concentration of carotenoids for those crabs that are highly parasitized. Thus, a lower concentration of carotenoids is seen in tissues from highly parasitized crabs. Furthermore, for the studies involving carapace tissue, crabs containing high amounts of β-carotene also had the highest mean parasite load; this could mean the parasites prevent the conversion of β-carotene to astaxanthin or that they selectively absorb astaxanthin from the carapace tissue. For ovarian tissue there was a higher level of parasitism for crabs that absorbed echinenone rather than β-carotene and zeaxanthin, indicating the parasites steal β-carotene and zeaxanthin from their intermediate host or that they select for crabs with high levels of echinenone.

## LITERATURE CITED:

Barret, J., and Butterworth, P. E. (1973). The carotenoid pigments of six species of adult acanthocephalans. *Specialia, 15(6):* 651-653.

Britton, G. (1995). UV/vis spectroscopy. *Carotenoids, 1B:* 13-62.

Cheesman, D. F., Lee, W. L., and Zagalsky, P. F. (1967). Carotenoproteins in invertebrates. *Biological Reviews, 42:* 132-160.

Crompton, D. W. T., and Nickol, B. B. (1985). *Biology of the Acanthocephala*. New York: Cambridge University Press. pg. 273-346.

Crompton, D. W. T., and Nickol, B. B. (1999). Reintroduction of Profilicollis Meyer, 1931, as a Genus in Acanthocephala: Significance of the Intermediate Host. *J. of Parasitology, 85(4):* 716-718.

Czeczuga, B. (1980). Carotenoids in fish. XI. Carotenoid level in tench, *Tinca tinca* (L.), affected by the presence of parasitizing *Eragsilus seiboldi* Nordm. (*Crustacea, Copepoda). Acta Parasitologica Polonica, 27:* 101-107.

Farallones Marine Sanctuary Association. (2002). *Acanthocephalan parasites: investigation in pacific mole crabs.* From the coastal ecosystem curriculum, San Francisco, CA.

Gilchrist, B. M., and Lee, W. L. (1971). Carotenoid pigments and their possible role in reproduction in the sand crab, *Emerita analoga* (Stimpson, 1857). *Comp. Biochem. Physiol., 42B:* 263-294.

Goodwin, T. W. (1980). *The biochemistry of the carotenoids volume II, 2nd ed*. New York: Chapman and Hall. pg. 64-96.

Hindsbro, O. (1972). Effects of *Polymorphus* (Acanthocephala) on colour and behaviour of *Gammarus lacustris. Nature, London, 238:* 333.

Lodish, H., Berk, A., Kaiser, C. A., Krieger, M., Scott, M. P., Bretscher, A., Ploegh, H., and Matsudaira, P. (2008). *Molecular Cell Biology, 6th ed*. New York: W. H. Freeman and Co. pg. 82.

MacGinitie, G. E. (1938). Movements and mating habits of the sand crab, *Emerita analoga. American Midland Naturalist, 19(2):* 471-481.

Ravishankar, G. A., Saraka, R., Usha, D., and Vidhyavathi R. (2006). An efficient method for extraction of astaxanthin from green algae *Haematococcus pluvialis. J. Agric. Food Chem. 54:* 7585-7588.

Schmidt, G. D., and Kuntz, R. E. (1967). Notes on the life cycle of *Polymorphus (Profilicollis) formosus* sp. n., and records of *Arhythmorhynchus hispidus* Van Cleave, 1925 (Acanthocephala) from Taiwan. *J. of Parasitiology, 53:* 805-809.

Shafaa, M. W. I., Diehl, H. A., and Socaciu, C. (2007). The solubilisation pattern of lutein, zeaxanthin, canthaxanthin and β-carotene differ characteristically in liposomes, liver microsomes and retinal epithelial cells. *Biophysical Chemistry, 129:* 111-119.

**APPENDIX**

*Note: A value of 1 was added to all parasite numbers and the log base ten of that number was taken. For the following tables the mean and standard deviation are both in log form. For each tissue, groups of 2 to 4 were divided based upon both natural breaks in the data and distinct wavelengths certain carotenoids maximally absorb.*

**Table 1**. Raw data for the UV/vis spectroscopy results on carapace tissue from *E. analoga*. ID number is the number assigned to the crab. The carapace length was obtained by measurement with digital calipers. The maximum absorbance and wavelength (nm) at that absorbance were obtained using Microsoft word. *Note: these samples were initially extracted using the ethyl ether procedure; the ethyl ether was evaporated off and the carotenoids were re-suspended in HPLC grade acetone. The average for each variable and the corresponding standard deviation are listed below, as obtained from Microsoft word.

| ID # | Carapace (mm) | Parasite Load | Max. Abs. | Wavelength at max. Abs. (nm) |
|---|---|---|---|---|
| 246* | 32.1 | 1 | 0.0879 | 466.0 |
| 248* | 25.2 | 0 | 0.3961 | 335.3 |
| 249* | 24.9 | 1 | 0.1600 | 466.0 |
| 251* | 20.3 | 0 | 0.4590 | 337.1 |
| 252* | 26.2 | 2 | 0.1903 | 466.0 |
| 254* | 25.6 | 1 | 0.2843 | 466.0 |
| 255* | 22.2 | 0 | 0.2370 | 466.0 |
| 260* | 23.3 | 1 | 0.3300 | 466.0 |
| 263 | 25.3 | 2 | 0.2928 | 480.2 |
| 268 | 24.7 | 0 | 0.3382 | 481.0 |
| 272 | 24.2 | 2 | 0.3286 | 480.2 |
| 278 | 25.3 | 1 | 0.4642 | 480.2 |
| 280 | 28.8 | 2 | 0.2493 | 480.2 |
| 283 | 26.1 | 2 | 0.3229 | 481.7 |
| 299 | 22.8 | 1 | 0.7545 | 481.7 |
| 318 | 22.9 | 2 | 0.2614 | 480.2 |
| 253* | 26 | 0 | 0.2437 | 486.3 |
| 261* | 24 | 0 | 0.4506 | 466.0 |
| 262* | 25.3 | 3 | 0.2331 | 466.0 |
| 271 | 25.3 | 0 | 0.4178 | 481.3 |
| 250* | 24.9 | 5 | 0.2080 | 486.3 |

| | | | | |
|---|---|---|---|---|
| 257* | 27.2 | 3 | 0.1627 | 466.0 |
| 259* | 25.7 | 5 | 0.1358 | 466.0 |
| 264 | 24.1 | 5 | 0.3463 | 481.7 |
| 266 | 25.8 | 4 | 0.3440 | 482.7 |
| 269 | 25.2 | 3 | 0.4066 | 479.2 |
| 275 | 29.6 | 5 | 0.2627 | 480.2 |
| 276 | 25.4 | 3 | 0.3120 | 479.2 |
| 277 | 30.4 | 5 | 0.2092 | 479.2 |
| 281 | 25.4 | 3 | 0.3318 | 479.2 |
| 301 | 24 | 3 | 0.3698 | 483.8 |
| 309 | 26.8 | 5 | 0.1817 | 479.2 |
| 310 | 26.6 | 3 | 0.3821 | 481.3 |
| 311 | 28.1 | 5 | 0.3594 | 479.2 |
| 315 | 26.7 | 3 | 0.1862 | 476.3 |
| 317 | 25 | 5 | 0.3049 | 481.7 |
| 313 | 23.8 | 5 | 0.2380 | 479.2 |
| 314 | 23.3 | 5 | 0.3379 | 481.0 |
| 265 | 25.9 | 8 | 0.4632 | 483.8 |
| 270 | 25.6 | 9 | 0.3875 | 479.2 |
| 273 | 25.3 | 11 | 0.3696 | 479.5 |
| 274 | 25.1 | 7 | 0.3680 | 479.2 |
| 279 | 30.5 | 7 | 0.1937 | 479.9 |
| 282 | 31.5 | 9 | 0.3400 | 475.3 |
| 300 | 29 | 8 | 0.2803 | 479.9 |
| 302 | 32.2 | 6 | 0.2647 | 483.1 |
| 303 | 26.2 | 8 | 0.2654 | 479.2 |
| 304 | 24.8 | 18 | 0.2756 | 482.0 |
| 305 | 25.4 | 15 | 0.2201 | 479.2 |
| 306 | 29.5 | 13 | 0.2974 | 481.7 |
| 307 | 26 | 22 | 0.2936 | 478.5 |
| 308 | 28.5 | 8 | 0.2509 | 481.3 |
| 316 | 26.9 | 14 | 0.2504 | 479.2 |
| 319 | 25.3 | 13 | 0.2601 | 483.8 |
| | | | | |

| | | | | |
|---|---|---|---|---|
| **Average:** | **25.5** | **3.1** | **0.3135** | **470.4** |
| **Standard Deviation:** | **02.4** | **4.8** | **0.1065** | **027.7** |

**Table 2**. Raw data for the UV/vis spectroscopy results on ovary tissue from *E. analoga*. ID number is the number assigned to the crab. The carapace length was obtained by measurement with digital calipers. The maximum absorbance and wavelength (nm) at that absorbance were obtained using Microsoft word. ***Note: this sample was too small to measure out the correct weight; a dilution was made to supplement. The volume was very small so a cube of paper wrapped in scotch tape with a height of 6mm was used at the bottom of the spectrophotometer to measure the absorbance. The average and standard deviation for each variable are displayed below.

| ID # | Carapace (mm) | Parasite Load | Max. Abs. | Wavelength at max. Abs. |
|---|---|---|---|---|
| 246 | 32.1 | 1 | 0.1668 | 454.6 |
| 248 | 25.2 | 0 | 0.6100 | 454.6 |
| 249 | 24.9 | 1 | 0.4026 | 454.6 |
| 251 | 20.3 | 0 | 0.4014 | 454.6 |
| 252 | 26.2 | 2 | 0.3801 | 454.6 |
| 254 | 25.6 | 1 | 0.2345 | 483.4 |
| 255 | 22.2 | 0 | 0.2287 | 454.6 |
| 263 | 25.3 | 2 | 0.1976 | 454.6 |
| 268 | 24.7 | 0 | 0.1682 | 454.6 |
| 272 | 24.2 | 2 | 0.2582 | 483.1 |
| 278 | 25.3 | 1 | 0.2222 | 454.6 |
| 280 | 28.8 | 2 | 0.2210 | 454.6 |
| 283 | 26.1 | 2 | 0.0906 | 483.4 |
| 247 | 24 | 4 | 0.1733 | 454.6 |
| 256 | 30.4 | 2 | 0.4429 | 454.6 |
| 258 | 25.2 | 1 | 0.3371 | 454.6 |
| 261 | 24 | 0 | 0.2493 | 471.0 |
| 276 | 25.4 | 3 | 0.1853 | 454.6 |
| 250 | 24.9 | 5 | 0.6207 | 454.6 |
| 257 | 27.2 | 3 | 0.1512 | 482.7 |
| 264 | 24.1 | 5 | 0.1564 | 454.6 |
| 266 | 25.8 | 4 | 0.3194 | 454.6 |

| | | | | |
|---|---|---|---|---|
| 269 | 25.2 | 3 | 0.1130 | 483.4 |
| 275 | 29.6 | 5 | 0.1904 | 454.6 |
| 277 | 30.4 | 5 | 0.1886 | 454.6 |
| 281 | 25.4 | 3 | 0.2364 | 483.8 |
| 301 | 24 | 3 | 0.1813 | 455.7 |
| 309 | 26.8 | 5 | 0.2998 | 456.5 |
| 310 | 26.6 | 3 | 0.0983 | 456.5 |
| 311 | 28.1 | 5 | 0.0817 | 456.5 |
| 315 | 26.7 | 3 | 0.0736 | 456.5 |
| 317 | 25 | 5 | 0.2419 | 456.5 |
| 318 | 22.9 | 2 | 0.2376 | 457.1 |
| 265 | 25.9 | 8 | 0.2137 | 456.4 |
| 270 | 25.6 | 9 | 0.2306 | 483.1 |
| 273 | 25.3 | 11 | 0.0786 | 454.6 |
| 274 | 25.1 | 7 | 0.1876 | 483.1 |
| 279 | 30.5 | 7 | 0.1392 | 482.7 |
| 282 | 31.5 | 9 | 0.1565 | 481.7 |
| 300 | 29 | 8 | 0.3049 | 456.1 |
| 303 | 26.2 | 8 | 0.2869 | 456.5 |
| 304 | 24.8 | 18 | 0.2009 | 456.1 |
| 305 | 25.4 | 15 | 0.1874 | 456.5 |
| 306 | 29.5 | 13 | 0.0836 | 456.9 |
| 307 | 26 | 22 | 0.2493 | 456.1 |
| 308 | 28.5 | 8 | 0.1710 | 456.1 |
| 319*** | 25.3 | 13 | 0.2570 | 452.5 |
| | | | | |
| **Average:** | **25.6** | **1.6** | **0.2942** | **460.0** |
| **Standard Deviation:** | **2.4** | **4.88** | **0.1193** | **011.6** |

**Table 3**. Raw data for the UV/vis spectroscopy results on egg tissue from *E. analoga*. ID number is the number assigned to the crab. The carapace length was obtained by measurement with digital calipers. The maximum absorbance and wavelength (nm) at that absorbance were obtained using Microsoft word. **Note: these crabs had brown, late stage, eggs; the rest of the eggs were orange, early stage, eggs. The extraction procedure was the same

for both. The average for each variable and the corresponding standard deviation are listed below, as obtained from Microsoft word.

| ID # | Carapace (mm) | Parasite Load | Max. Abs. | Wavelength at max. Abs. (nm) |
|---|---|---|---|---|
| 246 | 32.1 | 1 | 0.6771 | 458.2 |
| 248 | 25.2 | 0 | 0.8960 | 454.3 |
| 249 | 24.9 | 1 | 0.9950 | 454.6 |
| 251** | 20.3 | 0 | 0.8017 | 457.8 |
| 252 | 26.2 | 2 | 1.2330 | 454.3 |
| 254 | 25.6 | 1 | 1.0280 | 458.2 |
| 255 | 22.2 | 0 | 1.2030 | 454.6 |
| 260** | 23.3 | 1 | 0.4613 | 457.1 |
| 263** | 25.3 | 2 | 0.8941 | 456.7 |
| 268 | 24.7 | 0 | 0.8230 | 455.3 |
| 272** | 24.2 | 2 | 0.4047 | 456.4 |
| 278 | 25.3 | 1 | 0.4834 | 458.9 |
| 280 | 28.8 | 2 | 0.4271 | 483.1 |
| 283 | 26.1 | 2 | 0.8425 | 454.6 |
| 299 | 22.8 | 1 | 0.5121 | 458.9 |
| 318** | 22.9 | 2 | 0.5556 | 454.6 |
| 247** | 24 | 4 | 0.4933 | 458.9 |
| 253 | 26 | 0 | 0.6517 | 456.8 |
| 256 | 30.4 | 2 | 0.6840 | 454.6 |
| 258 | 25.2 | 1 | 0.6800 | 456.0 |
| 250 | 24.9 | 5 | 1.1000 | 458.2 |
| 257 | 27.2 | 3 | 0.6006 | 457.8 |
| 259 | 25.7 | 5 | 0.7500 | 458.2 |
| 264** | 24.1 | 5 | 0.4697 | 456.4 |
| 266** | 25.8 | 4 | 0.5467 | 455.7 |
| 269** | 25.2 | 3 | 0.4114 | 456.0 |
| 275 | 29.6 | 5 | 0.3955 | 457.1 |
| 276 | 25.4 | 3 | 0.8680 | 456.4 |
| 277 | 30.4 | 5 | 0.6956 | 457.8 |
| 281 | 25.4 | 3 | 0.2017 | 483.1 |

| | | | | |
|---|---|---|---|---|
| 301 | 24 | 3 | 0.8587 | 456.7 |
| 309** | 26.8 | 5 | 0.6294 | 456.0 |
| 310 | 26.6 | 3 | 1.1360 | 456.0 |
| 311 | 28.1 | 5 | 0.6531 | 454.6 |
| 315 | 26.7 | 3 | 0.7078 | 454.6 |
| 317** | 25 | 5 | 0.5816 | 456.0 |
| 261 | 24 | 0 | 0.6712 | 456.8 |
| 262** | 23.5 | 3 | 0.7564 | 457.1 |
| 267 | 21.3 | 0 | 0.6386 | 457.8 |
| 271 | 25.3 | 0 | 0.6690 | 457.8 |
| 265** | 25.9 | 8 | 0.5073 | 457.5 |
| 270** | 25.6 | 9 | 0.8176 | 456.7 |
| 273 | 25.3 | 11 | 0.5058 | 457.1 |
| 274 | 25.1 | 7 | 0.5067 | 456.4 |
| 279 | 30.5 | 7 | 0.9910 | 456.4 |
| 282 | 31.5 | 9 | 0.5085 | 458.2 |
| 300 | 29 | 8 | 0.5656 | 458.9 |
| 302 | 32.2 | 6 | 0.7581 | 456.4 |
| 303 | 26.2 | 8 | 0.4245 | 483.1 |
| 304 | 24.8 | 18 | 0.6197 | 456.4 |
| 305 | 25.4 | 15 | 0.6198 | 457.1 |
| 306 | 29.5 | 13 | 0.5823 | 456.0 |
| 307** | 26 | 22 | 0.5937 | 455.0 |
| 308 | 28.5 | 8 | 0.3859 | 456.0 |
| 316** | 26.9 | 14 | 0.7443 | 455.0 |
| 319** | 25.3 | 13 | 0.9749 | 456.0 |
| 313 | 23.8 | 5 | 0.6502 | 454.6 |
| 314 | 23.3 | 5 | 0.9250 | 453.9 |
| | | | | |
| **Average:** | **25.5** | **2.07** | **0.7103** | **457.5** |
| **Standard Deviation:** | **2.5** | **4.75** | **0.2204** | **006.1** |

**Table 4**. Raw data for the UV/vis spectroscopy results on esophagus tissue from *E. analoga*. ID number is the number assigned to the crab. The carapace length was obtained by measurement with digital calipers. The maximum

absorbance and wavelength (nm) at that absorbance were obtained using Microsoft word. The average for each variable and the corresponding standard deviation are listed below, as obtained from Microsoft word.

| ID # | Carapace (mm) | Parasite Load | Max. Abs. | Wavelength at max. Abs. |
|---|---|---|---|---|
| 246 | 32.1 | 1 | 0.7993 | 462.1 |
| 248 | 25.2 | 0 | 0.2874 | 462.1 |
| 249 | 24.9 | 1 | 0.3253 | 462.1 |
| 251 | 20.3 | 0 | 0.2501 | 477.0 |
| 252 | 26.2 | 2 | 0.3735 | 477.0 |
| 254 | 25.6 | 1 | 0.6596 | 481.0 |
| 255 | 22.2 | 0 | 0.2770 | 466.0 |
| 260 | 23.3 | 1 | 0.4830 | 466.0 |
| 263 | 25.3 | 2 | 0.2081 | 466.0 |
| 268 | 24.7 | 0 | 0.2480 | 466.0 |
| 272 | 24.2 | 2 | 0.3044 | 466.0 |
| 278 | 25.3 | 1 | 0.2792 | 466.0 |
| 280 | 28.8 | 2 | 0.3761 | 466.0 |
| 283 | 26.1 | 2 | 0.4193 | 466.0 |
| 299 | 22.8 | 1 | 0.2931 | 462.1 |
| 318 | 22.9 | 2 | 1.1910 | 478.5 |
| 247 | 24 | 4 | 0.3280 | 462.1 |
| 253 | 26 | 0 | 0.4987 | 479.5 |
| 256 | 30.4 | 2 | 1.0990 | 460.3 |
| 258 | 25.2 | 1 | 0.3666 | 466.0 |
| 250 | 24.9 | 5 | 0.4655 | 479.2 |
| 257 | 27.2 | 3 | 0.3934 | 466.0 |
| 259 | 25.7 | 5 | 0.6211 | 466.0 |
| 264 | 24.1 | 5 | 0.3644 | 466.0 |
| 266 | 25.8 | 4 | 0.5372 | 466.0 |
| 269 | 25.2 | 3 | 0.1011 | 466.0 |
| 275 | 29.6 | 5 | 0.3044 | 466.0 |
| 276 | 25.4 | 3 | 0.3870 | 466.0 |
| 277 | 30.4 | 5 | 0.7179 | 460.3 |
| 281 | 25.4 | 3 | 0.2130 | 466.0 |

| | | | | |
|---|---|---|---|---|
| 301 | 24 | 3 | 0.4237 | 462.1 |
| 309 | 26.8 | 5 | 0.3479 | 462.1 |
| 310 | 26.6 | 3 | 0.9143 | 477.8 |
| 311 | 28.1 | 5 | 0.6183 | 478.5 |
| 315 | 26.7 | 3 | 0.4724 | 462.1 |
| 317 | 25 | 5 | 0.5726 | 477.0 |
| 261 | 24 | 0 | 0.4748 | 476.3 |
| 262 | 23.5 | 3 | 0.5572 | 466.0 |
| 313 | 23.8 | 5 | 0.5189 | 462.1 |
| 265 | 25.9 | 8 | 0.4154 | 466.0 |
| 270 | 25.6 | 9 | 0.1943 | 466.0 |
| 273 | 25.3 | 11 | 0.3557 | 466.0 |
| 274 | 25.1 | 7 | 0.2644 | 466.0 |
| 279 | 30.5 | 7 | 0.7203 | 466.0 |
| 300 | 29 | 8 | 0.3926 | 462.1 |
| 302 | 32.2 | 6 | 1.2150 | 460.3 |
| 303 | 26.2 | 8 | 0.2851 | 478.8 |
| 304 | 24.8 | 18 | 0.7867 | 478.5 |
| 305 | 25.4 | 15 | 0.2790 | 477.8 |
| 306 | 29.5 | 13 | 0.3203 | 462.1 |
| 307 | 26 | 22 | 0.9041 | 478.8 |
| 308 | 28.5 | 8 | 0.5113 | 462.1 |
| 316 | 26.9 | 14 | 0.6394 | 477.0 |
| 319 | 25.3 | 13 | 0.6107 | 476.3 |
| | | | | |
| **Average:** | **25.3** | **1.5** | **0.4512** | **468.3** |
| **Standard Deviation:** | **2.4** | **4.8** | **0.2476** | **006.6** |

**Table 5.** Mean and standard deviation for groups of specific egg tissue absorption. Test 1 tested the hypothesis that the mean of group A was greater than that of group B. Test 2 tested the hypothesis that the mean of group A was less than that of group B. Group A included those specimens with wavelengths at maximum absorbance for egg tissue between 454 and 458 nm, which β-carotene absorbs. Group B included those wavelengths at maximum absorbance for egg tissue around 483 nm, characteristically absorbed by astaxanthin and canthaxanthin (Shafaa et al., 2007).

Note that both P values are significantly higher than either .05 or .01, indicating the null hypothesis cannot be rejected and that there is no significant difference between the parasite loads of the two groups.

| | ----------Test 1---------- | | | ----------Test 2---------- | | |
|---|---|---|---|---|---|---|
| **Group** | **Mean** | **St. Dev.** | **P-value** | **Mean** | **St. Dev.** | **P-value** |
| a | 0.615 | 0.369 | .752 | 0.615 | 0.369 | .376 |
| b | 0.677 | 0.248 | | 0.677 | 0.248 | |

**Table 6.** Mean and standard deviation for groups of specific carapace tissue absorption. Group A includes those carapaces which maximally absorbed around 475-476 nm, corresponding to canthaxanthin. Group B includes those specimens whose carapace maximally absorbed around 466 nm, also corresponding to canthaxanthin (Britton, 1995). Group C includes those specimens whose carapace maximally absorb around 480-485 nm, corresponding to both astaxanthin and canthaxanthin. Finally, group D includes those specimens whose carapace maximally absorb between 478 and 479.9 nm, corresponding to β-carotene (Czeczuga, 1980). Note that the P value is very small, indicating the null hypothesis can be rejected and therefore there is some significant difference between the parasite load of four distinct groups of maximal wavelength. Comparing the means and standard deviations this difference is present between all groups (see the bar graph following). This indicates that there is a difference in parasite load between the carapace tissues that absorb between 466 nm and 485 nm.

| **Group** | **Mean** | **St. Dev.** | **P-Value** |
|---|---|---|---|
| a | 1.84 | 0.647 | 0.0001 |
| b | 0.843* | 0.583* | |
| c | 1.45 | 0.811 | |
| d | 2.08* | 0.517* | |

*indicates significant difference

**Table 7.** Mean and standard deviation for groups of specific esophagus tissue absorption. Group A includes those crabs whose esophagus tissue absorbed at 460 nm, characteristic of echinenone and 4-hydroxy-4-keto-beta-carotene. Group B includes those crabs whose esophagus tissue absorbed at 462 nm, characteristic of echinenone (Britton, 1995). Group C includes the esophagus tissue that absorbed at 466 nm, characteristic of canthaxanthin. Finally,

group D includes the esophagus tissue that absorbed around 477-480 nm, characteristic of canthaxanthin, astaxanthin, and β-carotene (Czeczuga, 1980). Note that the P value is very high, indicating the null hypothesis will not be rejected and thus there is so significant difference in the parasite loads between these four groups of different esophageal wavelength absorption.

| **Group** | **Mean** | **St. Dev.** | **P-value** |
|---|---|---|---|
| a | 0.700 | 0.196 | .792 |
| b | 0.618 | 0.337 | |
| c | 0.595 | 0.290 | |
| d | 0.707 | 0.467 | |

**Table 8.** Mean and standard deviation for groups of specific ovarian tissue absorption. Group A indicates those specimens whose ovary tissue absorbed between 452-454 nm, representative of β-carotene and zeazanthin. Group B comprises the wavelengths of 455-457 nm, representative of echinenone. Group C comprises the wavelength of 470 nm, representative of astaxanthin. Finally, group D comprises the wavelengths between 480-483 nm, representative of canthaxanthin and astaxanthin (Britton, 1995). Note that the P value is smaller than 0.01 and 0.05, indicating the null hypothesis can be rejected and that there is some difference in parasite load between one or more groups. From the bar chart it is evident that groups B and C have significantly different parasite loads for those two groups of wavelength absorption as well as groups A and C, and C and D.

| Group | Mean | St. Dev. | P-Value |
|---|---|---|---|
| a | 0.497* | 0.340* | 0.001 |
| b | 0.895* | 0.269* | |
| c | 0.00* | -* | |
| d | 0.687* | 0.247* | |

*indicates a significant difference

*Note: For all graphs a value of 1 was added to the parasite load and the log base ten calculated*

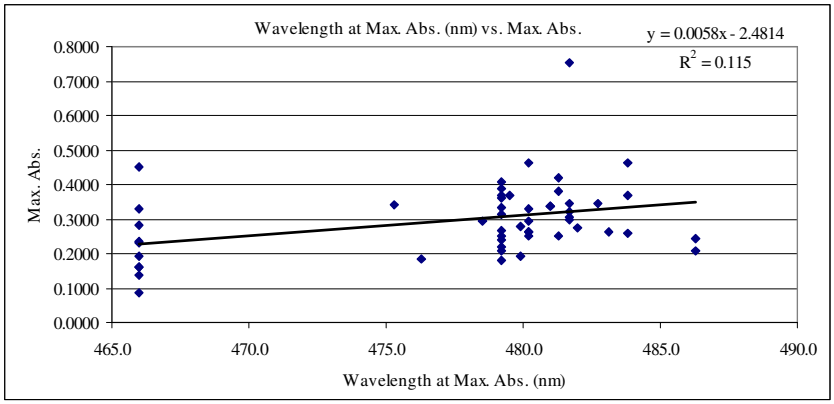


**Figure 1.1**. Wavelength at maximum absorbance (nm) vs. maximum absorbance for carapace samples. The equation for the line and the $R^2$ value are shown in the top right. Note that this graph excludes two outliers, crabs 248 and 251. See discussion for explanation.

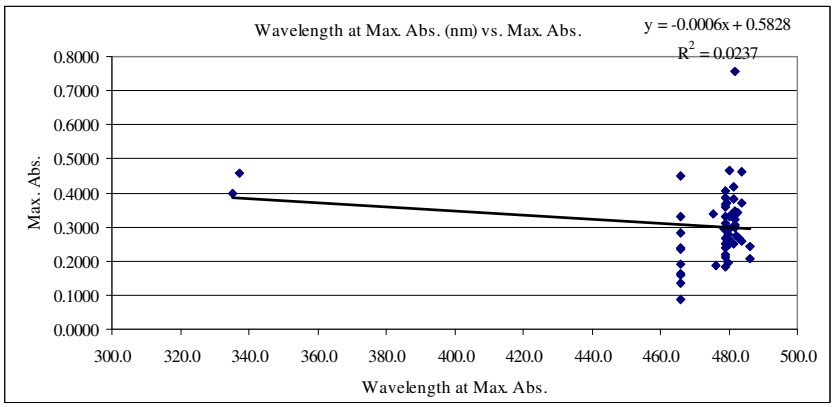


**Figure 1.2**. Wavelength at maximum absorbance (nm) vs. maximum absorbance for carapace samples. The equation for the line and the $R^2$ value are shown in the top right. Note that this graph includes all data from table 1. The two outliers on the far left are excluded in the above figure.

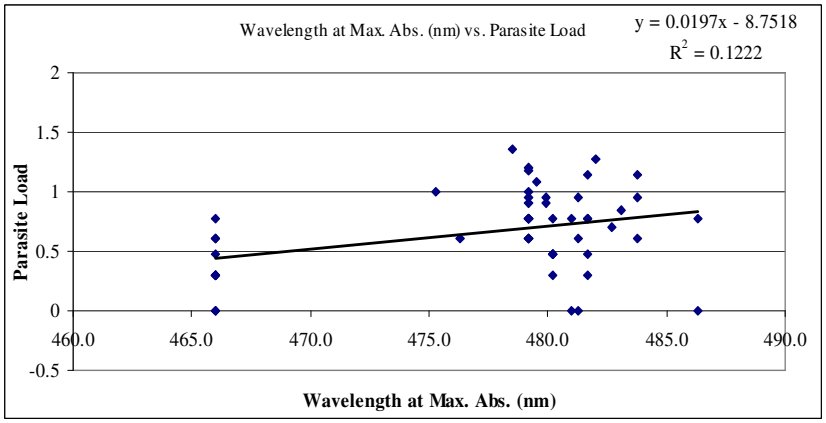


**Figure 1.3**. Wavelength at maximum absorbance (nm) vs. parasite load for carapace samples. The equation for the line and the $R^2$ value are shown in the top right. Note that this graph excludes the outliers, crabs 248 and 251, from table 1. See discussion for explanation.

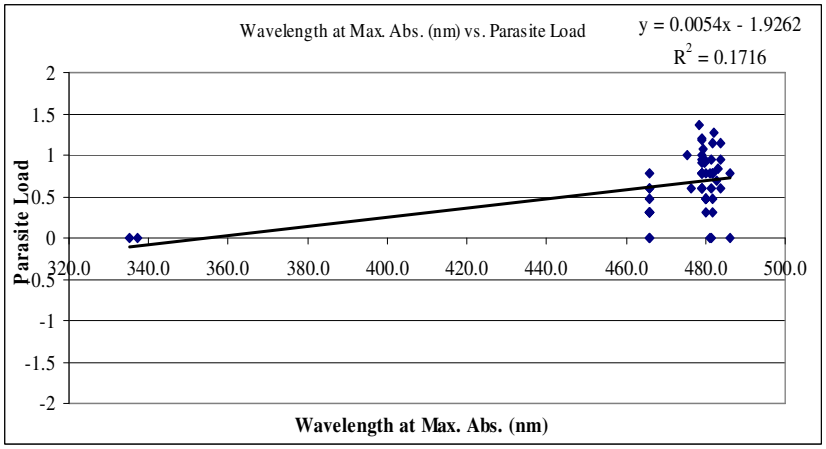


**Figure 1.4**. Wavelength at maximum absorbance (nm) vs. parasite load for carapace samples. The equation for the line and the $R^2$ value are shown in the top left. Note that this graph includes all data from table 1. The two outliers on the bottom left are excluded in the above figure.

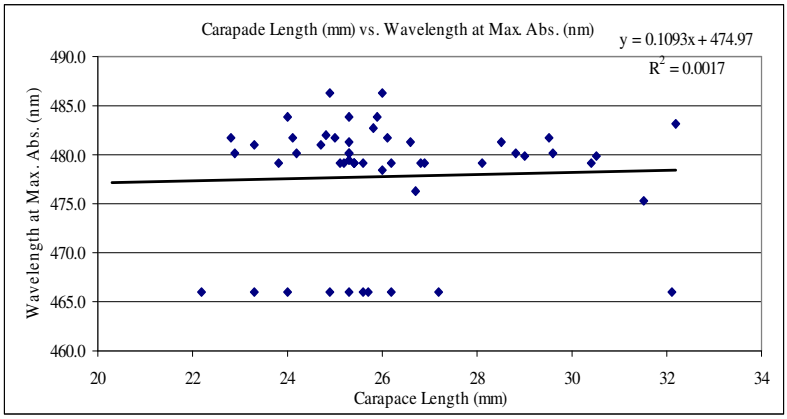


**Figure 1.5**. Carapace length (mm) vs. wavelength at maximum absorbance (nm) for carapace samples.. The equation for the line and the $R^2$ value are shown in the top right. Note that this graph excludes the two outliers, crabs 248 and 251, from table 1. See discussion for explanation.

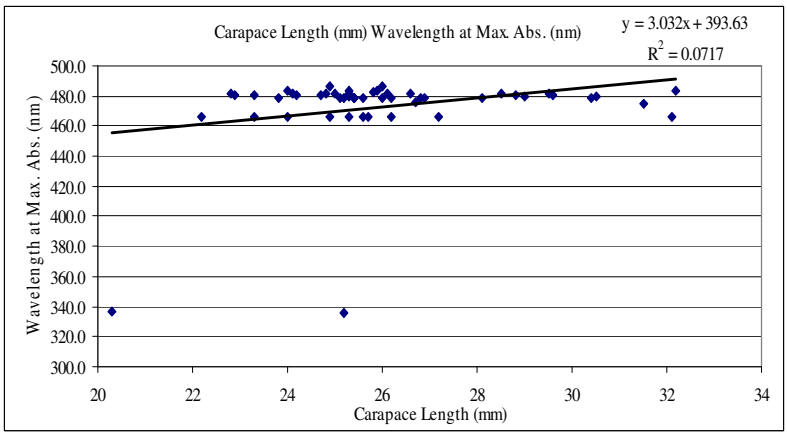


**Figure 1.6**. Carapace length (mm) vs. wavelength at maximum absorbance (nm) for carapace samples. The equation for the line and the $R^2$ value are shown in the top right. Note that this graph includes the two outliers shown in the bottom left that are excluded in the above figure.

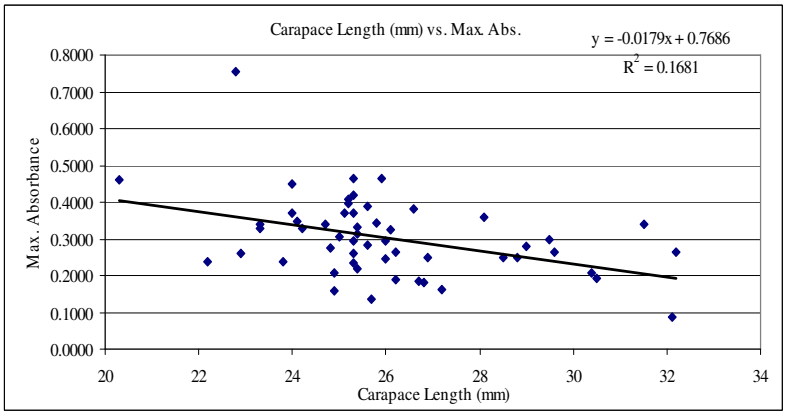


**Figure 1.7**. Carapace length (mm) vs. maximum absorbance for carapace samples. The equation for the line and the $R^2$ value are shown in the top right. Note that as size increases absorbance decreases.

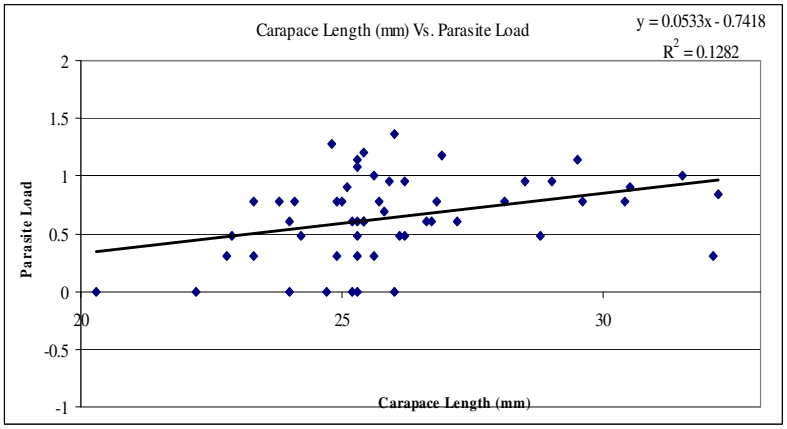


**Figure 1.8**. Carapace length (mm) vs. parasite load for all carapace samples. The equation for the line and the $R^2$ value are shown in the top right. Note that as carapace size increases so does parasite load.

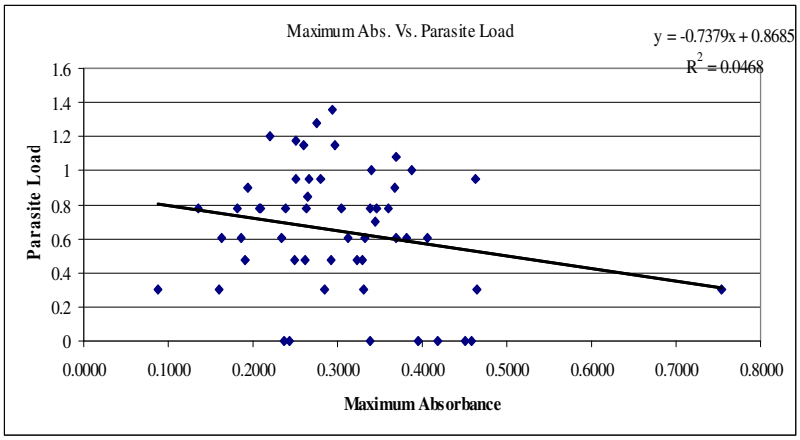


**Figure 1.9**. Maximum absorbance vs. parasite load for all carapace samples. The equation for the line and the $R^2$ value are shown in the top right. Note that as parasite load increases the maximum absorbance decreases.

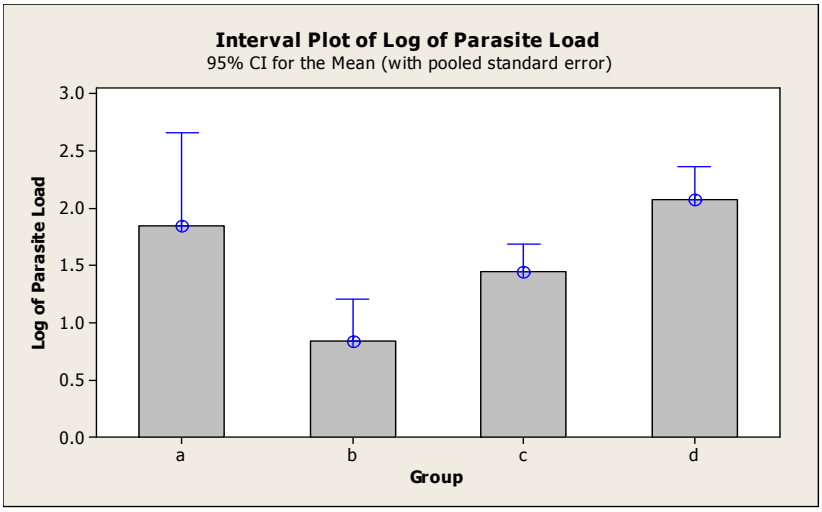


**Figure 1.10**. Based on wavelength absorption four separate groups of carapace tissue absorption were identified: a) 475-476 nm, b) 466 nm, c) 480-485 nm, and d) 478-479.9 nm. The mean and average standard deviation of parasite load is shown for each group.

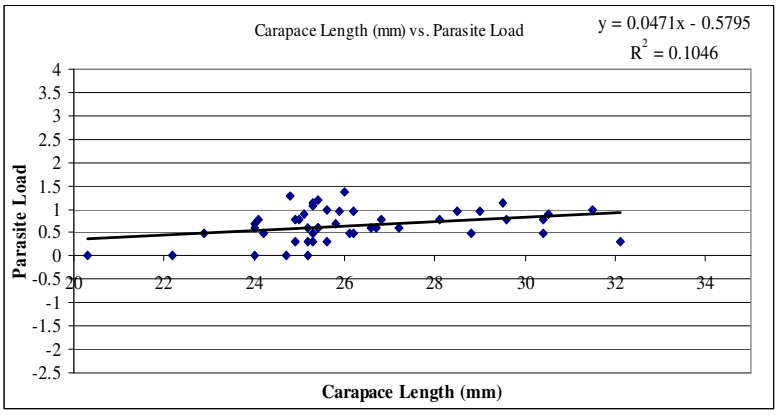


**Figure 2.1**. Carapace length (mm) vs. parasite load for all ovary samples. The equation for the line and the $R^2$ value are shown in the top right. Note again that as carapace length increases so does parasite load.

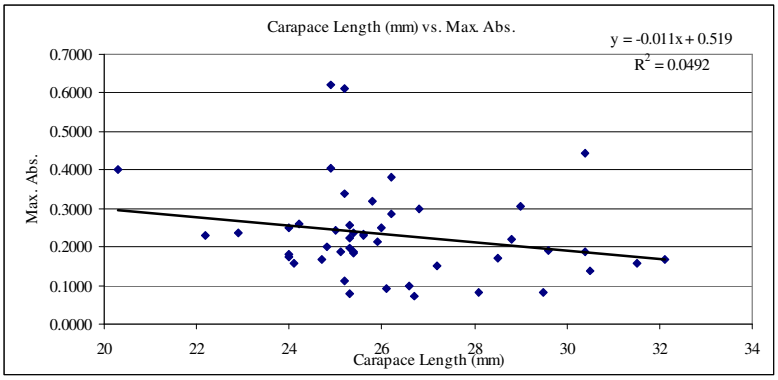


**Figure 2.2**. Carapace length (mm) vs. maximum absorbance for all ovary samples. The equation for the line and the $R^2$ value are shown in the top right.

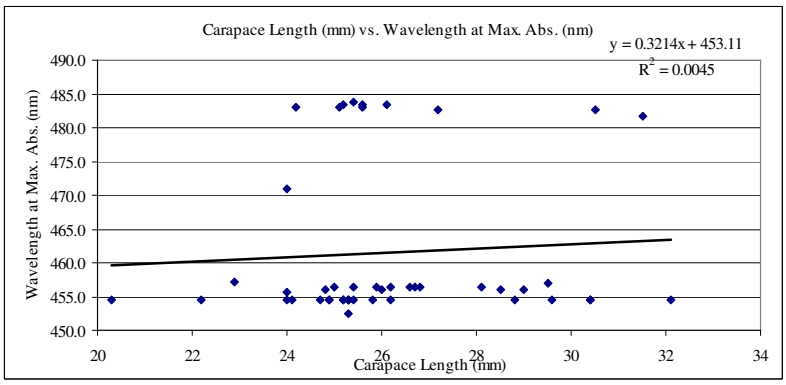


**Figure 2.3**. Carapace length (mm) vs. wavelength at maximum absorbance (nm) for all ovary samples. The equation for the line and the $R^2$ value are shown in the top right. Note that there are two distinct wavelengths at which the maximum absorbance is seen and in general the higher wavelengths correlate with a larger carapace.

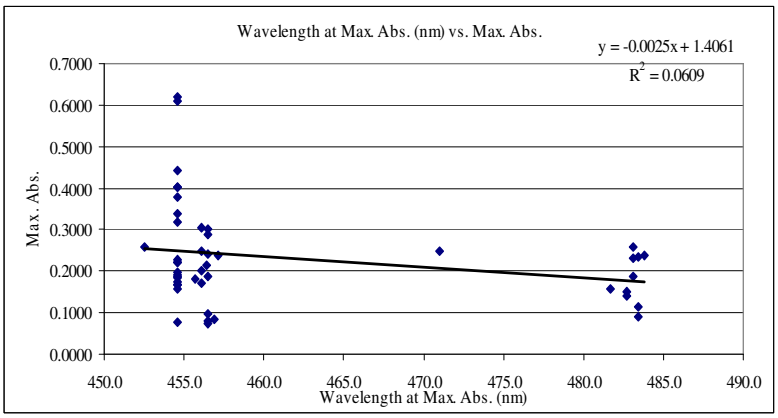


**Figure 2.4**. Wavelength at maximum absorbance (nm) vs. maximum absorbance for all ovary samples. The equation for the line and the $R^2$ value are shown in the top right. Note again that there are two characteristic wavelengths at which the samples absorb maximally.

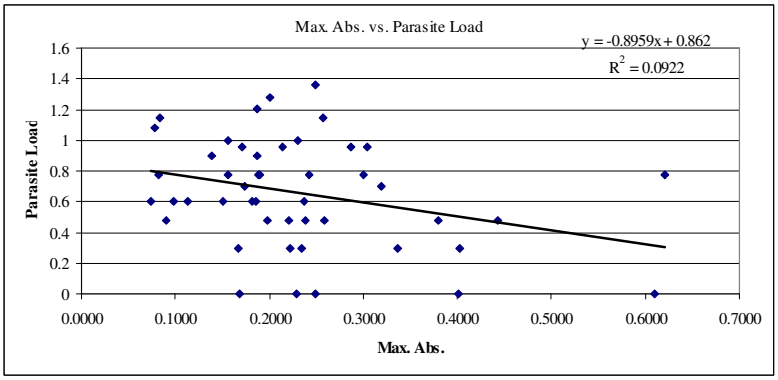


**Figure 2.5**. Maximum absorbance vs. parasite load for all ovary samples. The equation for the line and the $R^2$ value are shown in the top right. Note that as parasite load increases the maximum absorbance of the samples decreases.

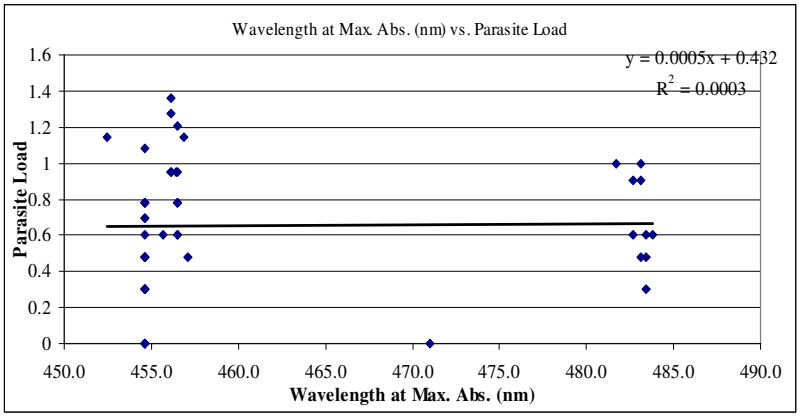


**Figure 2.6**. Wavelength at maximum absorbance (nm) vs. parasite load for all ovary samples. The equation for the line and the $R^2$ value are shown in the top right. Note again that there are two characteristic wavelengths at which ovary samples maximally absorb.

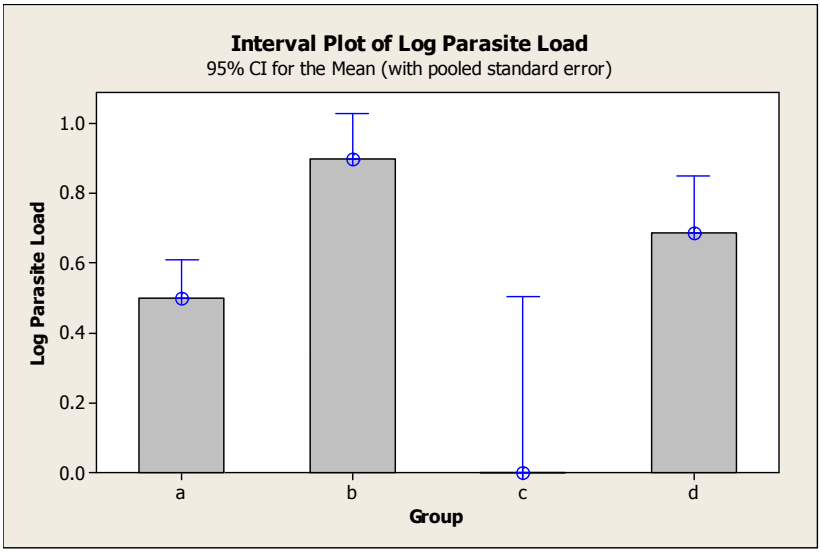


**Figure 2.7.** Based on wavelength absorption four separate groups of ovary tissue: 454-454 nm(a), 455-457 nm(b), 470 nm(c), and 480-483 nm(d). The mean and standard deviation of parasite load are shown. Note that group c has only one sample within the group.

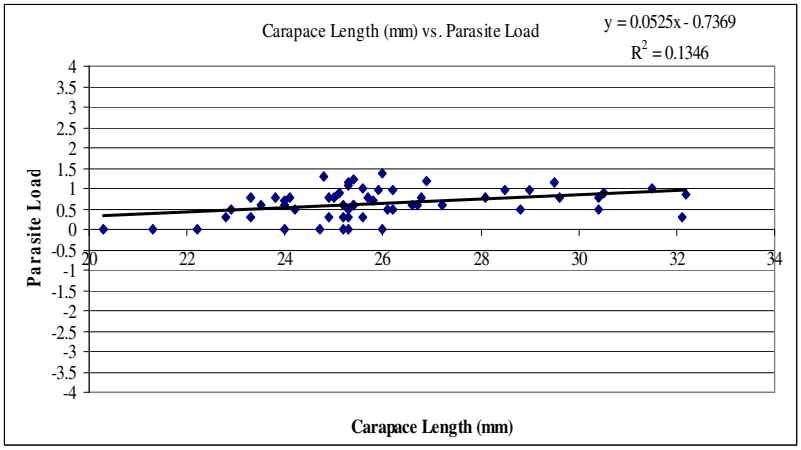


**Figure 3.1**. Carapace length (mm) vs. parasite load for all egg samples. The equation for the line and the $R^2$ value are shown in the top right. Note that as in other graphs as parasite load increases so does carapace length.

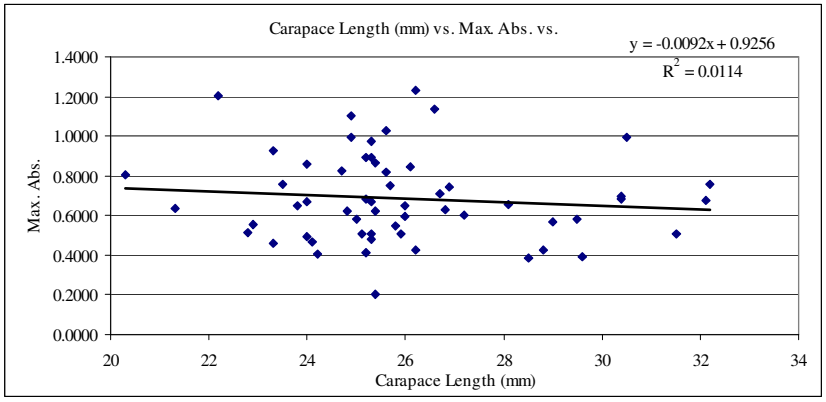


**Figure 3.2.** Carapace length (mm) vs. maximum absorbance for all egg samples. The equation for the line and the $R^2$ value are shown in the top right.

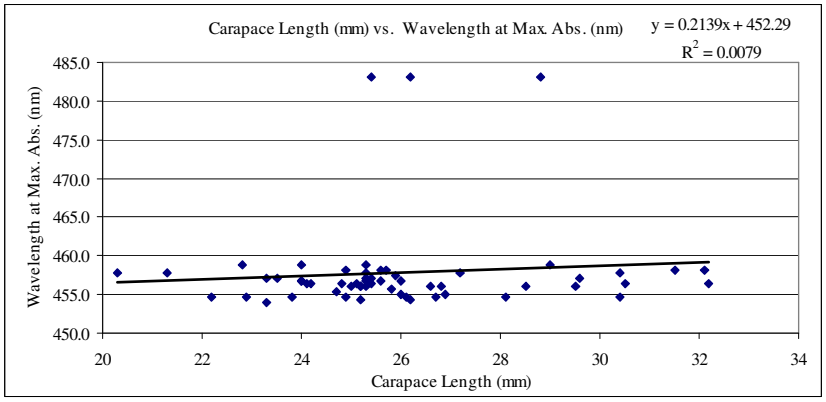


**Figure 3.3**. Carapace length (mm) vs. wavelength at maximum absorbance (nm) for all egg samples. The equation for the line and the $R^2$ value are shown in the top right. Note again that there are two distinct wavelengths at which egg samples maximally absorb.

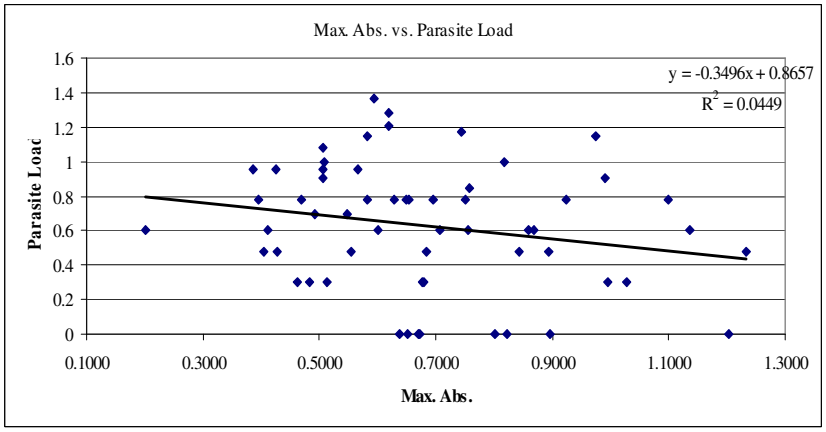


**Figure 3.4**. Maximum absorbance vs. parasite load for all egg samples. The equation for the line and the $R^2$ value are shown in the top right. Note as in other graphs as parasite load increases the maximum absorbance of the sample decreases.

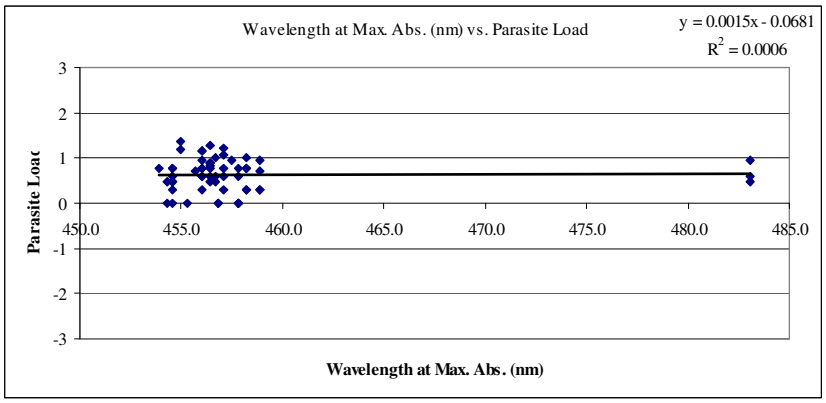


**Figure 3.5**. Wavelength at maximum absorbance (nm) vs. parasite load for all egg samples. The equation for the line and the $R^2$ value are shown in the top right. Note as in other graphs there are two distinct wavelengths at which samples maximally absorb. Unlike ovary samples the higher wavelength correlates with lower parasite load.

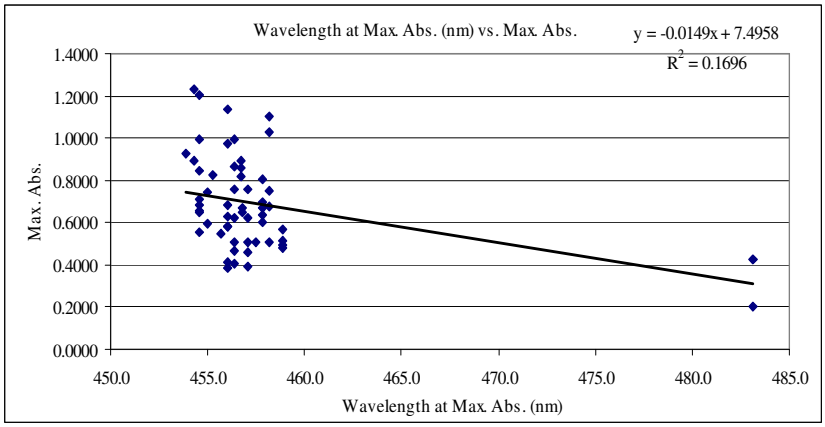


**Figure 3.6**. Maximum absorbance vs. wavelength at maximum absorbance (nm) for all egg samples. The equation for the line and the $R^2$ value are shown in the top right. Again, there are two distinct wavelengths at which egg samples maximally absorb.

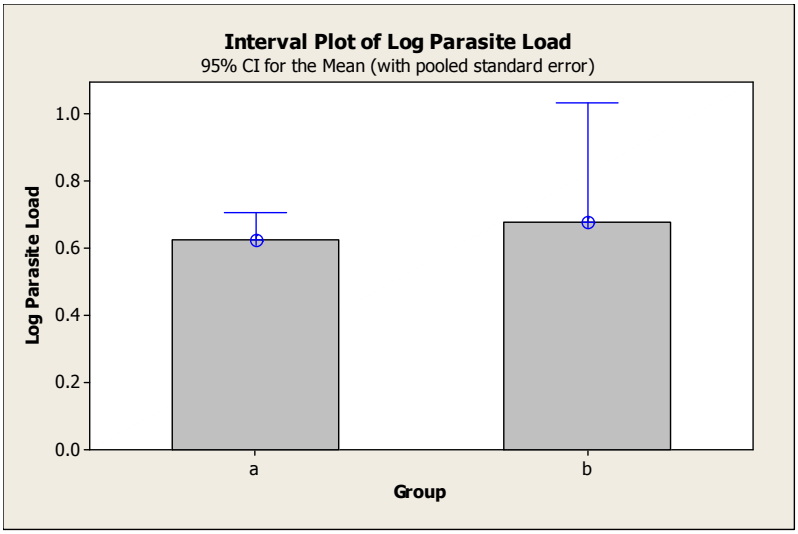


**Figure 3.7**. Values of wavelength at maximum absorbance naturally split into two groups: a) 454-458 nm and b) 483 nm. Shown on the right is their mean and standard deviation of parasite load.

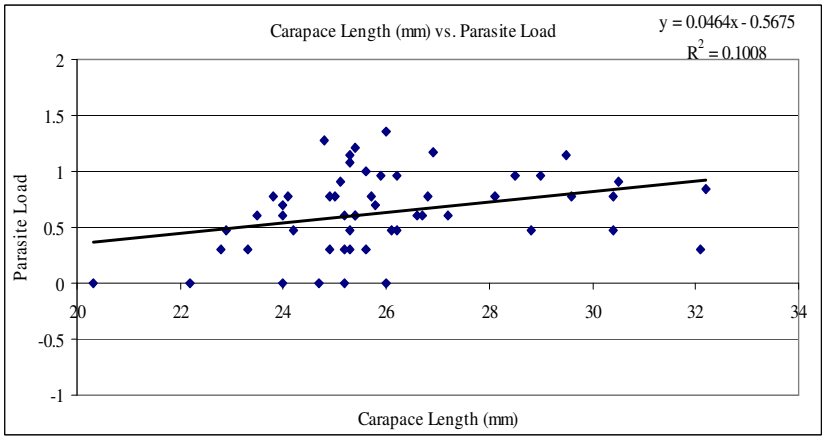


**Figure 4.1**. Carapace length (mm) vs. parasite load for all esophagus samples. The equation for the line and the $R^2$ value are shown in the top right. Note as before that as parasite load increases so does carapace length.

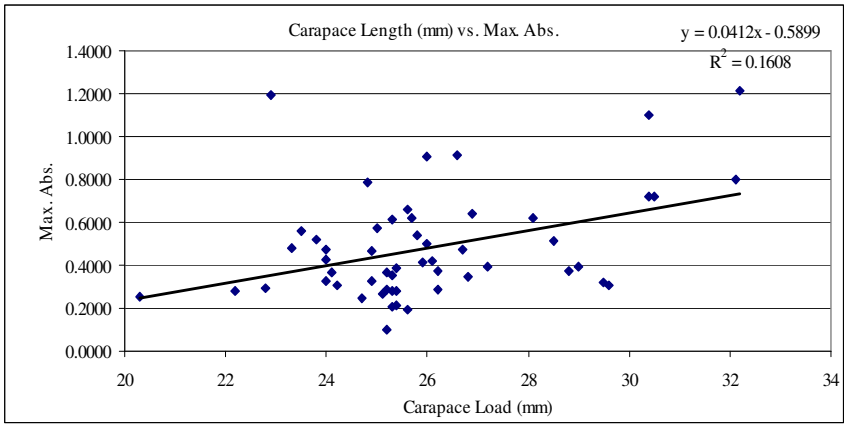


**Figure 4.2**. Maximum absorbance vs. carapace length (mm) for all esophagus samples. The equation for the line and the $R^2$ value are shown in the top right.

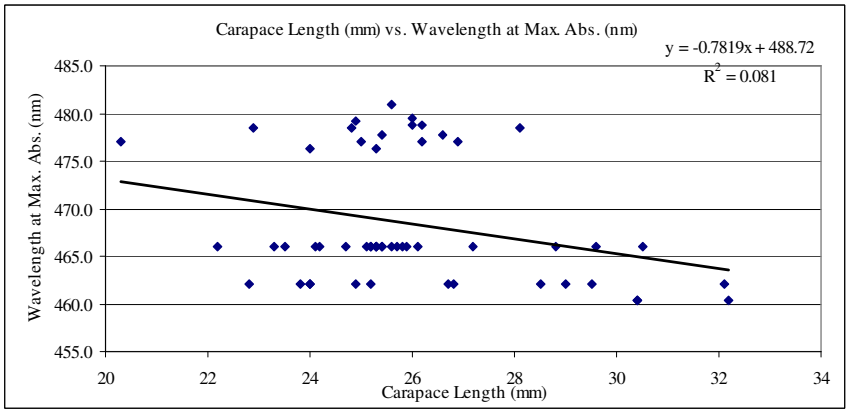


**Figure 4.3.** Wavelength at maximum absorbance (nm) vs. carapace length (mm) for all esophagus samples. The equation for the line and the $R^2$ value are shown in the top right.

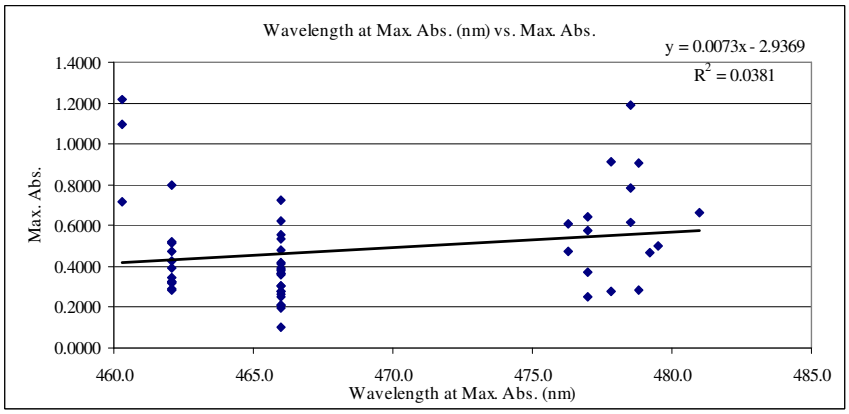


**Figure 4.4**. Maximum absorbance vs. wavelength at maximum absorbance (nm) for all esophagus samples. The equation for the line and the $R^2$ value are shown in the top right. Note that there are three to four characteristic wavelengths at which esophagus samples maximally absorb.

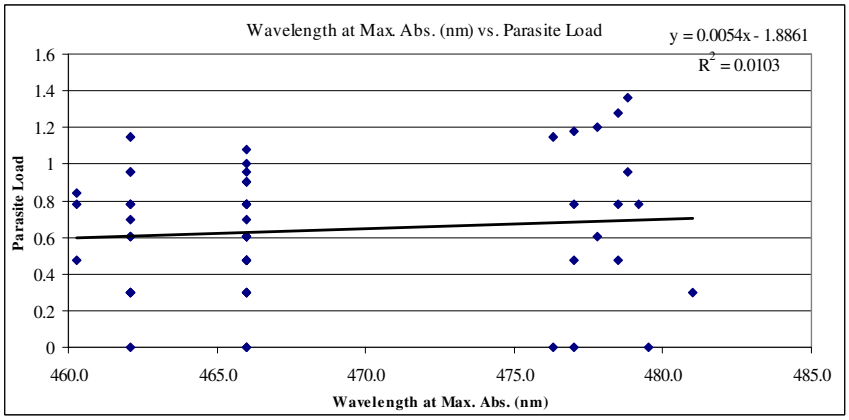


**Figure 4.5.** Wavelength at maximum absorbance (nm) vs. parasite load for all esophagus samples. The equation for the line and the $R^2$ value are shown in the top right.

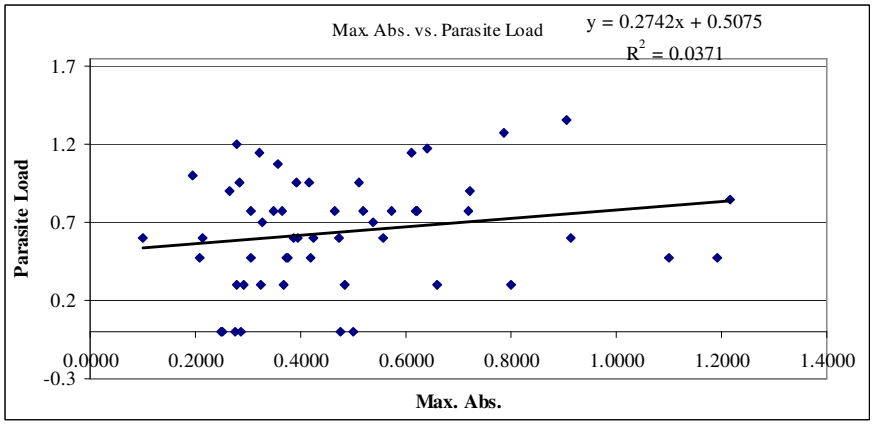


**Figure 4.6**. Maximum absorbance vs. parasite load for all esophagus samples. The equation for the line and the $R^2$ value are shown in the top right. Note that unlike the other graphs as parasite load increases the maximum absorbance increases as well. See discussion for hypothesis.

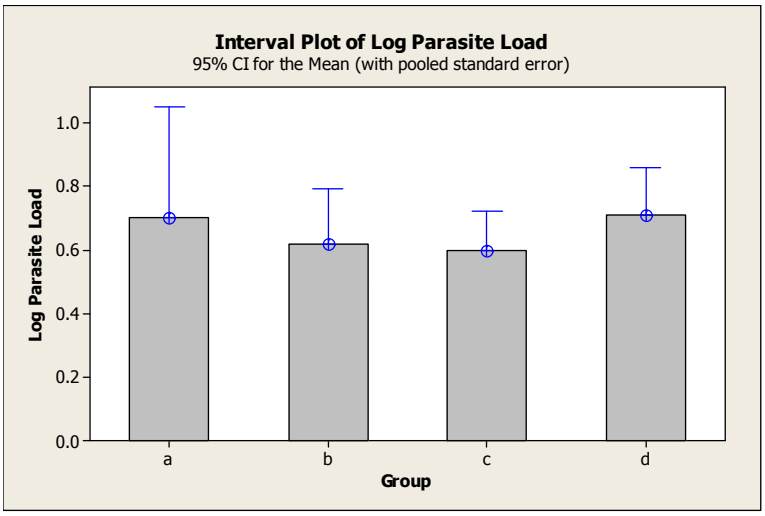


**Figure 4.7**. Four groups divided from the wavelength values of esophagus tissue: a) 460 nm, b) 462 nm, c) 466 nm, and d) 470-480 nm. The mean and standard deviation of parasite load are displayed on the graph.